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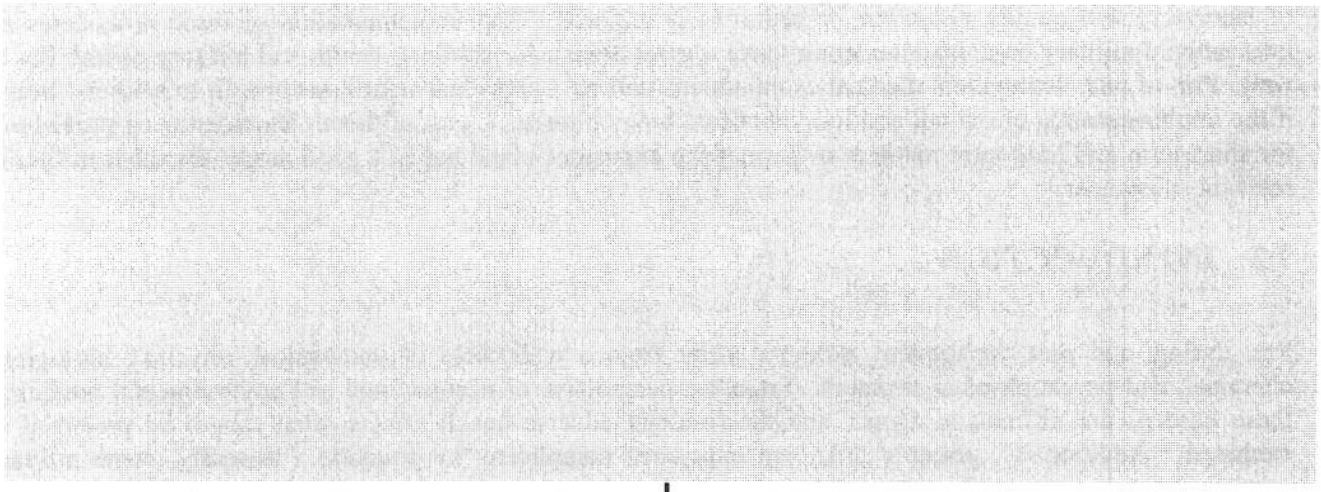
BIOTECHNOLOGIES FOR ASSESSMENT OF TOXIC HAZARDS IN OPERATIONAL ENVIRONMENTS



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Chapter 7 HUMAN EXPOSURE BIOMARKERS – PERMETHRIN AS A MILITARILY-RELEVANT MODEL

Klaus G. Mross[1], Gina E. Adam[2], Daan Noort[3], & Jeannot Zimmer[4]

[1]Bundeswehr Regional Medical Command II, Occupational and Environmental Medicine, Diez, Germany. [2]U.S. Army Research Institute of Environmental Medicine, Natick, MA, U.S.A.[3]TNO Defence, Security and Safety, Rijswijk, The Netherlands, [4]Bundeswehr Institute of Medical Occupational and Environmental Safety, Berlin, Germany

7.1 ABSTRACT

Soldiers in operational environments are exposed to a variety of chemical substances. During deployment, they often move very rapidly from one "environment" to the other. Therefore ambient monitoring is very difficult and its outcome very complex to assess. Just as occupational and environmental assessments are becoming more common in industry, biomonitoring of chemical substances and their metabolites in body fluids of soldiers is the best way to calculate individual exposures. Urine is the preferred choice, because it is easy available, can be produced on a regular basis and is simply processed for further analysis. For most of the exposures to militarily relevant substances, there exist laboratory methods to assess the soldiers' body burden. For distinct exposures, methods have to be developed, and additional scientific research is needed. These assays should be adopted in the military area, even when only the sample taking will occur in the deployment area and analysis and assessment will be done at home. Procedures for use in operational environments have to be introduced by national and international military institutions. International standards of laboratory and quality assurance procedures are required. The implementation of these procedures by international military organizations would have a great benefit for soldiers' health and military operability as well. Pre- or post-deployment medical examinations will only provide a significant benefit to soldiers' health if the implementation of overall and individualized biomonitoring is part of them. Biomonitoring procedures for short-term and long-term markers of permethrin exposures could act as a good model for other militarily relevant substances.

7.2 INTRODUCTION

Pre, during, and post deployment, soldiers suffer from a wide field of mechanical, physical, biological, chemical, and psychophysical stressors. From the perspective of occupational and environmental medicine, these burdens are difficult to assess, and the resultant adverse health effects often cannot be predicted or excluded. Additionally, possible unknown exposures complicate the situation. Recently, some military services have started conducting ambient monitoring during deployment. The interpretation of ambient monitoring during deployments is difficult for several reasons: first, it is essential to have an idea about what substances will be present at the right time and the right place; Second, deployed soldiers often move rapidly between unknown environments with additional danger of exposure. For that reason, additional measures have to be taken into consideration. As Kenneth Olden, Director of the *National Institute of Environmental Health* (NIEHS) stated: "Human exposure assessment is often the weakest link in risk assessment. … Adverse health outcome is a function of toxicity and exposure, both duration and intensity. … Environmental monitoring, which determines what's in air, soil, food, and water, is not equivalent to individual exposures."

[1]. Thus, individual biomonitoring is essential, yet we must still determine what could and should be measured when, how, why, and what benefit this provides for deployed soldiers. Soldiers are often exposed to unknown chemicals. But more often, they are exposed to well known "home made" substances, such as fuels, explosives, lubricants, pesticides etc. Biomonitoring can provide accurate information regarding exposure to chemical substances. Used properly, it may play an important role in military operational decision-making to benefit the soldier health.

7.3 HUMAN BIOMONITORING

Whereas ambient monitoring covers the amount and fate of chemical substances in the environment and on workplaces, biological monitoring is the determination of the levels of presumably toxic substances or their metabolites in body fluids or exhaled air. Determining their metabolites, adducts with proteins and DNA or other products of intermediate metabolism defines the contact and/or impact of these substances on the individual. Biological monitoring is a valuable way to protect an individual against the adverse health effects of chemicals in the workplace or in the environment. For that reason, it is necessary to implement a system of monitoring that allows assessments of exposures. These assessments may be either "ambient" or "biological" monitoring. Both should not be regarded as opposite. They should be considered complementary.

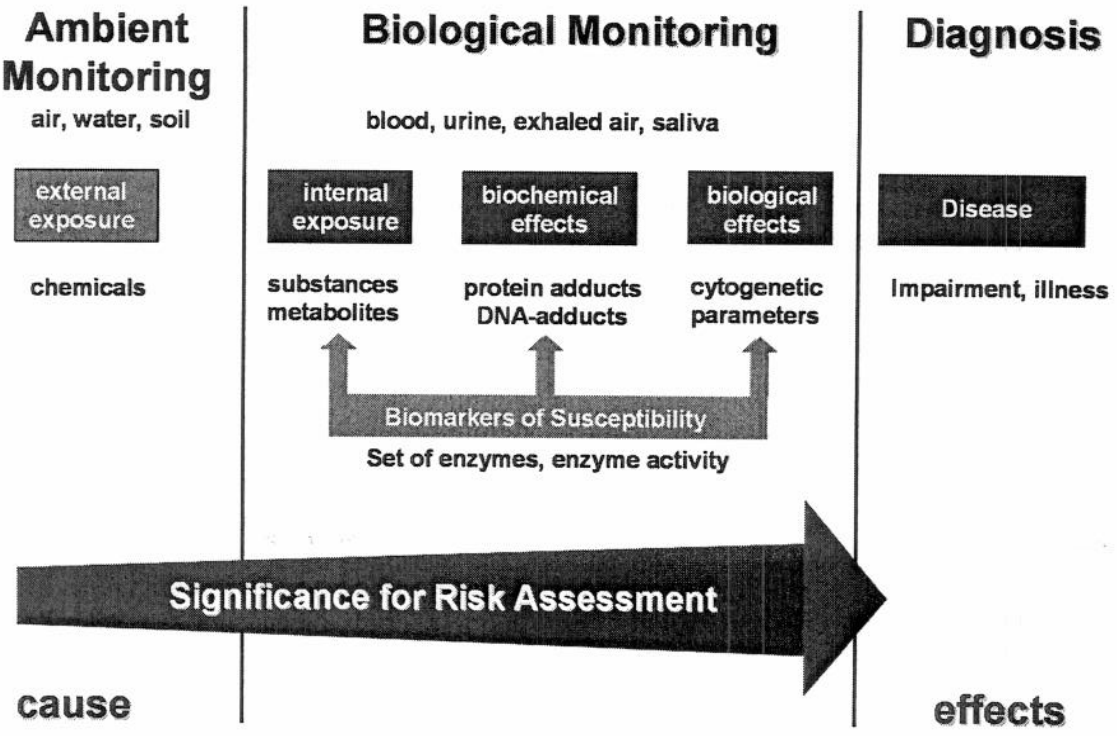

Figure 1: Monitoring chemicals in occupational and environmental sciences (modified from [2])

Biological monitoring focuses on the measurement of interactions between chemical substances and the human "biological system". According to the *International Programme on Chemical Safety* (IPCS) three classes of biomarkers can be identified [3]:

- Biomarkers of Exposure — An exogenous substance or its metabolite or the product of an interaction between a xenobiotic agent and some target molecule or cell that is measured in a compartment within an organism,

- Biomarkers of Effect — A measurable biochemical, physiological, behavioural or other alteration within an organism that, depending upon the magnitude, can be recognized as associated with an established or possible health impairment or disease (for an extensive overview see Chapter 2),

- Biomarkers of Susceptibility — An indicator of an inherent or acquired ability of an organism to respond to the challenge of exposure to a specific xenobiotic substance (for an extensive overview see Chapter 8).

Biochemical effects, and to an even greater extent biological effects, are closer to the ultimate damaging principle and thus to the effects of the toxic substance than is dose monitoring. This means the predictive value of biological monitoring parameters increases from dose monitoring to effect monitoring. However, at the same time, the substance specificity decreases towards effect monitoring. With many effect parameters, such as chromosomal aberrations or DNA strand breaks, it can no longer be determined whether they occurred as a result of exposure to specific substances. In biomonitoring of exposure, this is quite different. Here the toxic substance can be determined specifically, but without providing any direct information about the effects [2,3-5].

The goal of biological monitoring is to improve the prevention of diseases caused by toxic substances and their metabolites. Relationships must be established between parameters of biological monitoring and indicators of early stages of diseases. Exposed persons and groups of the general population should be investigated. Epidemiological expertise is required during planning of studies and the evaluation of results. In this context, according to Angerer, the following can be regarded as biological monitoring parameters [2-5]: concentration of substance and/or its metabolites in body fluids, toxic products of intermediary metabolism, forming of protein and DNA adducts, impacts on genotypes and phenotypes, changes of immunological parameters, cytogenetic parameters, and early toxic effects on susceptible target organs (see chapter 2 and 8). For this purpose, analytical procedures should be drawn up and validated such that further biomarkers of exposure, effect, and individual susceptibility will be determined. Furthermore, there must be progress in the preparation of biological material and enrichment of these products. New methods of instrumental analysis must be integrated into biological monitoring. Not just using ICP-MS and LC/MSMS, but also to techniques such as PCR-techniques with subsequent MS-detection. It should be guaranteed in all studies that the data collected are comparable. To achieve this, existing biological monitoring procedures must be validated and their reproducibility checked [2].

Biomonitoring procedures have become increasingly important in occupational and environmental health risk assessments.

Especially during deployment, standardization procedures must be carried out in the preanalytical phase.

One of the main problems during military deployments is the storage and transportation of samples under correct temperature conditions in time.

7.3.1 Biomarkers of Exposure

7.3.1.1 Present Applications

Biomonitoring of exposure is used to confirm and assess the exposure of individuals or populations to a particular substance, providing a link between external exposures and internal dosimetry. After its introduction in the 1930's, biological monitoring in occupational and environmental medicine increased steadily since the 1960's, closely linked with the progress in analytical techniques and the growing knowledge of metabolic pathways [2]. Since that time, it has been an ever growing important tool in medical health surveillance in the European Union and the United States. In Germany, since 1975 the DFG *Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area* has promoted the development of suitable, valid and tested analytical methods for biomarkers of exposure. Meanwhile, a collection of analytical methods has been produced that is like no other world-wide. In 1979, the DFG commission began to evaluate tolerable threshold limit values for the parameters of biological monitoring, such as *Biological Tolerance Values for Occupational Exposures* (BAT) and *Exposure Equivalents for Carcinogenic Substances* (EKA) [6]. In 1982, the *German Society of Occupational and Environmental Medicine* began to check the quality of the results of biological monitoring in intercomparison programs [7]. Today, an extensive intercomparison program for occupational and environmental toxicological routines is organized in Germany. In fact, more than 150 biomarkers of exposure (chemicals or their metabolites) can be detected by validated procedures in different body fluids or exhaled air [8]. Unlike in many other countries, biological monitoring is mandatory due to legal regulations on health and safety at work. For this reason there is an extended practical experience in the use of biological monitoring of exposure in occupational medicine. Biological monitoring of exposure is applied to environmental toxicology as well, which is shown by epidemiological studies world-wide [9-12].

7.3.1.2 Main Prospects

In view of the international IPCS working group: "biological markers of exposure pose unique advantages as tools for multimedia exposure assessment. They are highly sensitive indices of an individual's exposure to chemicals, since they provide a measure of the internal dose, account for all routes of exposure and integrate over a variety of sources of exposure. Therefore, they can represent past exposure (e.g., the presence of lead in shed teeth), recent exposure to an external source (e.g., volatile organic compounds in exhaled breath) and even future internal exposure sources (e.g., pesticides in adipose tissue). Furthermore, their use results in improved monitoring of total population exposure, characterization of individual and population exposures and evaluation of internal sources of exposure (see [12]). These markers are also useful surveillance tools for monitoring chemical exposure in both individuals and populations over time. Use of biological markers of exposure can improve the risk assessment process by providing a critical link between chemical exposure, internal dose and health impairment" [5].

The measurable parameters of biological monitoring are modulated by interindividual differences in detoxifying a substance: Enzyme polymorphisms, occurrence or absence of distinct enzymes or the metabolising capacities are the reason for differences in the performance of xenobiotic metabolizing enzymes. (see Chapter 8).

When absorption mainly occurs through skin or when individual protective devices are used, biomarkers of exposure can provide reliable measurements of internal dose, which are useful to assess dose-response relationships.

The rational use of biomarkers of exposure needs sufficient toxicological knowledge about the substances toxicokinetics (absorption, absorption route, distribution, elimination, or may be accumulation), metabolism, their mode of action and adverse health effects. Different kinetic aspects, sometimes integrated in a physiologically based pharmacokinetic model (PBPK) help to define an appropriate biological monitoring strategy.

7.3.1.3 Integrated Access

Identification of suitable biomarkers associated with different toxic end-points or outcomes requires the collaboration of different scientific disciplines such as toxicology, occupational medicine, environmental medicine, molecular genetics, analytical and synthetic chemistry, and epidemiology. Only a small number of highly specialized scientists work in the area of exposure to toxic substances. Therefore international collaboration is necessary. Epidemiological studies should incorporate biological monitoring wherever possible. Epidemiology investigates relationships between exposures, biochemical, biological, and health effects. The description of the exposure situation according to the yes/no principle or with scores has proved to be unsuitable in many studies. Studies in which data for dose monitoring, effect monitoring, and health effects can be linked using epidemiological methods are urgently needed. With such a relationship, the advantages and disadvantages of the individual biological monitoring parameters can be exploited or compensated for. By identifying the parameters with the greatest diagnostic validity it should be possible not only to improve the prevention of health effects, but at the same time to limit the efforts needed. By determining the dose taken up or the resulting biochemical and biological effects, epidemiological investigations may provide more precise information about health effects. With the use of epidemiological methods in biological monitoring it should be possible to clarify pathomechanisms, to recognize the early stages of diseases, and also to include the genetically determined susceptibility in the estimation of the risk. However, if epidemiological methods are to be used in biological monitoring, the methodological shortcomings often observed (validity of the test, power of the study, selection of the control group etc.) must be excluded [2,5]. New sciences like computational toxicology offer additional ways of supporting this integrated access.

7.3.1.4 Quality Assurance

Successful research in the field of biological monitoring needs analytical results comparable from laboratory to laboratory. This means that conditions must be created which lead to the minimization of possible influences on the analytical result. In the preanalytical phase all conditions for preanalytical procedures must be standardized and well documented. Main problems are the selection of appropriate biological media and sampling time. The analytical phase must be accompanied by an effective internal and external quality control. Important prerequisites are, for example, reference and standard substances and control materials. The availability of reference substances represents an ever growing problem for biological monitoring. Chemists involved in the preparation of samples should therefore be included in the research in this area. Detailed descriptions of the complete analytical procedures, so-called standard operating procedures (SOPs), lead to the further reduction of possible influences on the analytical result [2,5].

As far as the quality control of the analytical procedure in this field is concerned, the most progress has been made in dose monitoring (see above). In case of biochemical effect markers and biological effect markers,

however, development is still at its beginning. Therefore, these activities must be included in appropriate research projects. The validity and reproducibility of all analytical procedures used in exposure biomonitoring must be checked according to the criteria of national or international standardization institutions, such as the German DFG commission [2-5,8].

7.3.1.5 Future Needs

By determining toxicants and their metabolites in body fluids and exhaled air, the dose taken up can be evaluated specifically and sensitively (internal exposure, dose monitoring). Due to the methods of instrumental analysis available today, it is possible to detect many substances down to the concentration ranges relevant to environmental exposures. The spectrum of parameters today includes metals, organic solvents, pesticides, aromatic amines and aromatic nitro compounds, polyaromatic hydrocarbons, etc. Nevertheless, this parameter spectrum must be continuously extended. In this context, the human metabolism of many organic substances, such as pesticides, must be clarified. The aim is to find those metabolites which are closest to the health risk. With the help of metabolic profiling, in addition to the ultimate toxic metabolite, interindividually different susceptibilities should be recognizable. Indispensable for both dose monitoring and the determination of protein and DNA adducts is progress in the sector of instrumental chemical analysis. ICP-MS should be included in the determination of metals. This method is suitable for the simultaneous detection of groups of refractory elements. The technological combination HPLC and multiple MS provides new measurement possibilities which are needed for the determination of adducts, specifically and sensitively, which have been enriched by prior handling with augmentation techniques like PCR. This requires the use of tailor-made chromatographic separation phases such as *restricted access material* (RAM) or *molecular imprinting* (MIP) [2,5].

7.3.2 Military Applications

Measurement of environmental exposures (i.e. ambient and biological monitoring) is essential for risk assessment and risk management. It is even more important for deployed military forces in uncharacterised (unknown) environments. The major goal of risk assessment is to prevent and predict disease from chemical or other environmental exposures. Until approximately 20 years ago, calculating an external exposure and assuming its correlation to the internal exposure determined the risk associated with environmental exposures. Today, there is an essential need to obtain pre-deployment and post-deployment biomonitoring data from military personnel. New strategies have to be developed in order to meet these new requirements, which should include the following points: knowledge base (Information sampling), process planning, coordination, praeanalytics, transport, laboratory procedures, risk assessment, and reaction / countermeasures. Interoperability between the scientific specialities mentioned above, not only medical, is ultimately needed. It must be noticed that this process is time-critical. It should be well prepared and conducted under strict planning. During bi/multinational deployments, international scientific relations should be enhanced with bi/multinational access to the results. This saves time and resources and will bring much more benefit to the individual soldiers of all participating nations.

7.4 PERMETHRIN

7.4.1 Physical and Chemical Properties

Permethrin, chemically 3-Phenoxybenzyl(1RS;3RS)-3-2,2-dichlorvinyl-2,2dimethylcyclo-propancarboxylate, CAS-Nr C 52645-53-1, has a molecular weight of 391.3 g/mol. It is a clear light brown liquid with a weak

characteristic odour. Melting point: 55.7-56.3°C (cis), 45.7-46.3°C (trans), solubility in water: 0.07mg/l (25°C), density: 1.0138 (25°C), vapour pressure: 2.15×10^{-8} mmHg (cis), 0.69×10^{-8} mmHg (trans). The industrial purity is approx. 90-99% [13].

7.4.2 Occurrence

Permethrin does not occur naturally. Like all pyrethroids, it is a synthetic analogue of the chrysanthemic acid (aliphatic ester compound from pyrethrum). The substance is used predominantly in agriculture. The quantity used in agriculture is approx. 10-20 g/ha (compared with several hundred g/ha in the case of other insecticides). For preventive medicine purposes, permethrin is used in different applications, such as fogging and spraying operations, impregnation of bed nets in malaria control operations etc. It is for sale to outdoor enthusiasts at commercial outlets world wide under different brands. Its application has been favoured in almost all external and internal areas of vector control because of its distinct lower toxicity to mammals, than to insects. Permethrin is more effective against a broad range of pests, than the more toxic organochloro, organophosphate, and carbamide insecticides. Because of its widespread use in agriculture, its occurence in humans is ubiquitous. Biomonitoring studies showed detectable permethrin metabolites in urine at very low concentrations within the general population in different countries [9-12,14].

Environmental and toxicological qualities of permethrin are known from animal experiments and human exposures. They are collected and updated regularly in different free available databases like *Hazard Substance Databank* (HSDB) and *Integrated Risk Information System* (IRIS) [15,16]. The substance is reviewed by international and national agencies, like the *U.S. Agency for Toxic Substances and Disease Registry* (ATSDR), *International Programme on Chemical Safety* (IPCS) and the *World Health Organization* (WHO) [13,17-19].

7.4.3 Military Use

There are a variety of applications for permethrin in the military area. Several armies in the world use pesticide combinations to protect their troops from disease vectors such as mosquitoes, sand flies, fleas, ticks, mites and lice. It is also important to shield the soldiers from the distraction and discomfort of biting bugs which impacts soldier morale. A complete review of usage and policies in military services has not been completed. A complete review on a standardized basis, to be updated permanently is recommended.

The ***U.S. Army*** currently uses three methods for adding permethrin to uniforms. Two of these require the individual soldier to apply a solution to his uniform. The first of these is the *spray can procedure* developed in the 1980's which has a concentration of 0.5% and lasts for 5 or 6 launderings. This method involves a 6 oz. aerosol can which is used to spray a hanging uniform (i.e. not while on soldier's body, nor is the spray to be applied directly to skin) and then the uniform is allowed to dry. The second method is the *Individual Dynamic Absorption Application* (IDAA) kit which includes plastic bags and two 9 ml bottles of permethrin in 40% concentration, each of which will treat one uniform piece. The procedure requires the user to combine ¾ canteen of water and one bottle of permethrin in one locking plastic bag, shake bag to mix chemical, add the uniform piece (BDU pants or top), close bag and shake, and then leave to soak for 2 ½ hours or more; after which the soldier removes the garment from the bag and hangs to dry (product label). A third method is to be performed by trained personnel using a 2-gallon sprayer to apply 151 mL of permethrin at 40% concentration in two gallons of water for 50 seconds on each side to uniforms that are lying on the ground. This product is also applicable to bed netting. For both the IDAA kit and the 2-gallon sprayer application, the operator is required to wear protective gloves (the sprayer also requires a respirator: product label, MSDS). In both of these applications, after the uniform is hung to dry for three hours, no further application is necessary and

permethrin protection is estimated to last 50 launderings, considered the life of the BDU. Additionally, instructions for all of the procedures state that uniform surfaces that touch the skin, such as underwear and headgear, are not to be treated. In addition to the methods outlined above which require the individual soldiers to treat their uniforms, the Army has developed an industrial application method. This method is the "preferred and recommended method" and is available through approved contractors in the commercial sector and provides for treated uniforms that the soldiers could buy or be issued. At this point, the factory impregnation with permethrin is available for woodland-pattern Battle Dress Uniforms (BDU's) and the desert camouflage uniform and has a permethrin treatment level of 0.125 mg per square centimeter. This treatment is also designed to last the life of the uniform, approximately 50 washings. To date, these uniforms are not yet available for distribution or purchase, subject to approval by the Army Uniform Board.

Current Army policy regarding the use of permethrin for deployed troops is held in the U.S. Army Center for Health Promotion and Preventive Medicine (CHPPM) instruction letter for protection from biting insects. This guidance states that the best protection from biting insects (and thus, vector-borne diseases) is to use the DoD Insect Repellent System. This system is composed of three items: 1) a permethrin-treated uniform, 2) DEET on exposed skin surfaces, and 3) proper wear of the uniform (to include tucking in of undershirt and pants legs, no rolling of sleeves). The policy further guides soldiers to sleep under permethrin-treated bed nets and take all malaria prophylaxis pills as directed. These guidelines direct soldiers to use either the spray can or IDAA kit for permethrin treatment of their uniforms. Although the factory treatment procedure appears to be available, it does not seem to have been put into practice. In fact, an Armed Forces Pest Management Board (AFPMB) Information Paper states that no uniforms of any type have been factory-treated and distributed.

According to anecdotal reports from individuals who have been recently deployed to Iraq or Afghanistan, soldiers are being required at the CONUS Replacement Center (CRC) to pre-treat their uniforms either with the IDAA kit or spray can before leaving for the deployment theater. These individuals also reported that they did not see any evidence of permethrin treatment re-supply while they were in theater.

Safety analyses include: extensive animal studies; an Army-commissioned Committee on Toxicology report; an AFPMB Information Paper; a Health Hazard Assessments performed by/for the Army; and a risk/benefit analysis performed comparing risk of malaria/leishmaniasis to the toxicity of permethrin/chemical exposure. As for the safety and efficacy of permethrin treated uniforms, Army policy is that permethrin is effective in limiting biting insects and that there are no safety issues to soldiers wearing treated uniforms. Additionally, there are permethrin, deltamethrin, and cyfluthrin treatments available for impregnating bed nets or for spraying tents and curtains.

Current army policies, guidelines and safety analyses may be viewed and downloaded from the homepages of *U.S. Army Center for Health Promotion and Preventive Medicine* (CHPPM) or the *U.S. Armed Forces Pest Management Board* (AFPMB) [20,21].

Faulde reported on the ***German Armed Forces*** approach which is highly similar to the US approach to include the recommendations that soldiers wear a permethrin treated uniform, use repellents like diethyltoluamide (DEET) on exposed skin, and wear the uniform properly. A recently developed factory based treatment method for the uniforms has been available since 2002. Faulde et al tested this permethrin polymer-coated uniform (Producer UTEXBel). It was found that uniforms impregnated in this way were life-time effective, ensuring protection of soldiers in the field from arthropod vectors, while showing less cross-contamination than those treated by the Insect/Arthropode repellent fabric treatment [22-24].

7.4.4 Toxicokinetics

Like all other pyrethroids, permethrin is lipophilic and has a very low vapour pressure. It is primarily absorbed by inhalation, generally by airborne dust; to a lesser extent through skin. Skin exposure may be predominant in pesticide operators. The general public is mostly exposed to permethrin residues in food and textiles [13,17,18].

After absorption, permethrin is rapidly distributed throughout the body, mainly in the adipose tissue, stomach, intestine, liver, kidneys and the nervous system [17].

Once distributed, permethrin is rapidly and extensively detoxified by ester hydrolysis and hydroxylation in blood, liver, and other organs including significant amounts in the nervous system [12,16]. Main metabolites are 3-phenoxy benzyl alcohol, further oxidized to 3-phenoxy benzoic acid (3-PBA), 3-(4'hydroxy)-phenoxybenzoic acid (4-OH)3-PBA and the two stereoisomers cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (cis-DCCA) and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (trans-DCCA) [13,17].

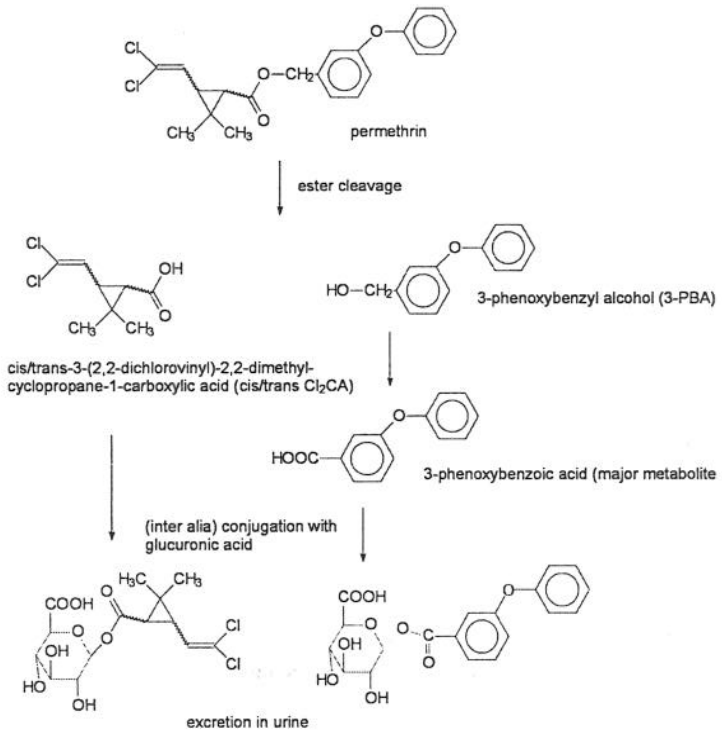

Figure 2: Metabolism of Permethrin

After conjugation, the metabolites are excreted as glucuronides, sulphates or acetates primarily via the kidneys. A small amount is eliminated in the faeces [13]. The elimination time is biphasic, with a first phase within hours, whereas the second phase lasts for several days, indicating slower elimination from adipose

tissues. Excretion normally ends within five days after terminating exposure, but can be delayed depending on the amount absorbed, route of exposure and individual factors of susceptibility [17].

7.4.5. Health Effects

Permethrin has been used for many years, with no major symptoms of human poisoning reported [17,19]. It is characterized by a moderate acute toxicity and does not show any evidence of long-term toxicity in humans [19]. No indication exists that permethrin has significant adverse effects on humans when used as recommended. It has induced skin sensations and paraesthesia in exposed workers, but these effects disappeared within 24 hours. Transient numbness, itching, tingling, and burning sensations have been reported in a small percentage of humans after dermal exposure to permethrin when it was used to treat head lice. Permethrin is not sensitizing to the skin, but may be slightly irritating to skin, eyes and nose [17].

Almost all ***systemic effects*** resulting from exposure to permethrin are related to the action of permethrin on the nervous system. It exerts its profound effect by prolonging the open phase of the sodium channel gates when a nerve cell is excited. Neurological signs typically result from acute toxicity. Low-level chronic exposure usually does not cause neurological signs in mammals, largely because of rapid metabolism and elimination. Data from animal studies do not indicate that permethrin significantly affects end points other than the nervous system, although changes in liver weight and metabolism sometimes have been used as an index of adverse effect levels. A few recent animal studies indicate the potential for adverse neurodevelopmental effects at dose levels at which other effects have not been reported. It should be considered in the interpretation of the neurotoxic effects observed in neonatal mice, consisting of changes in the density of muscarinic receptors at low doses: The unclear biological significance of the observed findings; the differences of mouse brain development process to that of humans, and the lack of standardization and comparability of the methods applied in neurotoxicological studies. Based on these considerations, further investigation of neurotoxicity is needed before conclusion can be drawn on this subject [19]. Data do not indicate that permethrin should be considered a carcinogenic concern to humans [17]. No data in humans are available regarding the potential for permethrin to cross the placental barrier and enter a developing fetus [17]. Permethrin was not mutagenic in the Ames test and was negative in two reverse mutation tests in *Escherichia coli* [13].

Studies of laboratory animals exposed to permethrin are summarized with *Non Observed Adverse Effect Levels* (NOAEL) and *Lowest Observed Adverse Effect Levels* (LOAEL) indicated [13,15-19].

Standards and Guidelines

World Health Organization (WHO) drinking water guideline	20 µg/L [13]
Food and Agriculture Organization/WHO accepted daily intake (ADI)	0.05 mg/kg BW/day [15]
Environmental Protection Agency Reference Dose (RfD)	0.05 mg/kg BW/day [16]

7.4.6 Permethrin Biomonitoring of Exposure

7.4.6.1 Short Term Markers of Exposure

There are two main approaches to evaluating human exposure to permethrin. The first is by determining the unchanged substance in plasma or serum and the second by determining its metabolites in urine. These two laboratory procedures reveal different information about permethrin exposure. Leng et al. report that urinary metabolites are the detoxified part of the pyrethroids and thus metabolite levels provide information regarding

the magnitude of exposure. Conversely, plasma or serum determinations can indicate unchanged pesticide levels which could be related to symptoms. They further suggest that permethrin should only be determined in plasma for a few hours after a significant exposure. They have not found a correlation between metabolite concentrations in urine and symptoms after exposure in pest control operators. Some of their work is looking for a way to determine pyrethroid susceptibility. Their work seems to suggest that the half-life for an individual's metabolism (esterase activity) is related to symptoms and that half-life is increased if there is a co-exposure [25,26].

The urinary metabolites serve as biomarkers of low and extremely low exposures and have been confirmed to be valid. Pyrethroids degrade to 3-phenoxybenzoic acid (3-PBA) and other metabolites, such as cis-3-(2,2-dichlorovinyl)- 2,2-dimethylcyclopropane-l-carboxylic acid and trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane- l-carboxylic acid (cis-CI_2CA and trans-CI_2CA), cis- 3 -(2, 2-dibromovinyl)- 2,2-dimethylcyclopropanol- carboxylic acid (cis-Br_2CA), and 4-fluoro-3-phenoxybenzoic acid (F-PBA). Main permethrin metabolites are cis-DCCA, trans-DCCA, and 3-PBA, which are also metabolites for other pyrethroids. Therefore, Br_2CA and F-PBA is sometimes done to differentiate a permethrin exposure from that of deltamethrin and cyfluthrin. This assessment is easily conducted as all five metabolites may be determined in one laboratory run [27,28]. Further it is possible to assess the exposure route (dermal, inhalation/oral) by calculating the ratio of DCCA and 3-PBA and the ratio of the DCCA stereoisomers [26,29]. The development of analytical procedures for the measurement of the above mentioned metabolites led to the method of Schettgen et al. using capillary gas chromatography with mass-selective detection (GC-MS) [27]. This method provides accurate detection of concentrations in urine for environmental exposures, but requires several hours to achieve acceptable detection levels. In military environments different levels of exposure exist, and it is difficult to detect exposures at the lowest levels. By introducing the LC/MSMS techniques into the routine, the detection level is increased and a broader range of concentrations can be determined within minutes. Also this procedure allows processing of a greater number of samples for screening purposes. Another benefit is the more preserving hydrolysis of samples by using glucuronidases than concentrated acids.

7.4.6.2 Longer term Markers of Exposure

In contrast to the above methods, Noort et al. propose a different method for measuring exposure to pyrethroids. They suggest that since urine metabolites have a short half-life and thus clear the body quickly, a different method should be used to evaluate long-term or chronic exposure to pyrethroids. They posit that a further step in the metabolism of pyrethroids involves the formation of adducts with protein in plasma. They suggest that evaluation of albumin would provide a measure of *chronic* exposure to permethrin, something which cannot be evaluated via urine samples. It is well known that protein adducts of xenobiotics represent a much more persistent class of biomarkers than metabolites excreted into urine, having half-lives up to several weeks or months. For instance, protein adducts in human tissues have provided mechanistic insight into the epidemiological associations between smoking and cancer [30]. Accordingly, Noort et al have developed methods for biomonitoring of exposure to CW agents based on mass spectrometric analysis of such protein adducts, e.g., adducts of sulfur mustard with hemoglobin and albumin and of adducts of nerve agents with butyrylcholinesterase (for an overview see [31]). In view of its chemical structure, it should not be expected that permethrin itself will react with proteins to produce adducts. An ongoing study is focussed on the (presumed) protein adducts of glucuronides of the two major carboxylic acid metabolites of permethrin, i.e., 3-PBA and cis/trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-1-carboxylic acid (DCCA; see Figure 2). Results have not yet been published [32]. Such adducts may serve as cumulative biomarkers for chronic exposure to permethrin. The O-acyl glucuronides represent a unique class of electrophilic metabolites, capable of reaction with nucleophilic sites in proteins. Numerous examples of these reactions have been documented in which the O-acyl glucuronides originated from drugs having a carboxylic acid moiety, such as

several non-steroid anti-inflammatory drugs (NSAID's), lipid lowering agents (gemfibrozil, clofibric acid), diuretic agents (furosemide) and the antiepileptic drug valproic acid [33]; (see 34] for an extensive overview). McKinnon and Dickinson [35] investigated the persistence of adducts of diflunisal- and probenecid-glucuronides with plasma proteins in volunteers. The adducts were still measurable at least one month after the parent drugs were undetectable. Several of the above-mentioned NSAIDS (e.g., benoxaprofen, zomepirac, ibufenac, tolmetin), have been withdrawn from the market because of severe adverse effects in patients, possibly due to antibody formation against the adducts with proteins, especially in the liver and kidneys [34]. Conjugation to glucuronic acid ("glucuronidation") by UDP-glucuronosyltransferase-mediated transfer of a glucuronyl moiety of UDP-glucuronic acid to a nucleophilic site of a xenobiotic is one of the major Phase II detoxicification reactions. It renders the xenobiotic more polar which facilitates its excretion. This reaction takes place predominantly in the liver [36]. The most likely candidate for protein adduct formation by acyl glucuronides is human serum albumin (HSA), which is a rather abundant protein in the plasma (see, e.g., [37,38]). It has been demonstrated that the lysine 195 and 199 residues in the hydrophobic pocket of subdomain IIA of HSA are preferentially modified by various acyl glucuronides [39-41]. Adducts to lysine residues are probably rather stable in vivo. Interestingly, it was recently shown that these particular lysine residues are also highly reactive towards the acylating agent phosgene [35]. In this case an intramolecular adduct was formed, in which the lysine 195 and 199 residues were bridged intramolecularly by a urea-type chemical bond (with the carbonyl moiety derived from phosgene). Protein binding of glucuronides of benzoic acids that are structurally related to 3-PBA has been reported (see, *e.g.*, [42]). In more general terms, it appears that the degree of covalent binding to proteins of acidic drugs in man correlates well with the chemical reactivity of the glucuronides of these drugs [33]. It can be derived from the available data that adduct formation with proteins of glucuronide derivatives of carboxylic acid metabolites of permethrin is probable and will provide a useful biomarker to assess cumulative exposure to this pyrethroid. During preliminary experiments it was indeed found that the β-glucuronide derivatives of 3-PBA and DCCA are reactive towards several model peptides, including glutathione. In future work, it will be investigated whether similar adducts are formed in vivo, and whether they can be used for biomonitoring purposes (see also Chapter 10).

7.4.6.3 Bundeswehr Approach to Permethrin Biomonitoring of Exposure

Starting a risk assessment with a literature review focused on dermal permethrin absorption (for an extended overview on dermal absorption see [43]), several washing and cross contamination tests and intensive efficacy tests against different arthropods were conducted [22-24]. As a result Bundeswehr decided to supply BDUs impregnated with the above mentioned UTEXBel procedure to troops deployed to areas with high vector risks. For the estimation of exposure Snodgrass predicted from dermal absorption studies in rabbits at a concentration of 0.125 mg/cm^2 in the fabric, a dermal permethrin transfer very far below the ADI of 0.05 mg/kg BW per day [44]. The *Subcommittee to Review on Permethrin Toxicity from Military Uniforms* recommended conducting additional absorption and metabolism studies in small groups of soldiers [45]. Following this advice, the German Armed Forces Medical Corps conducted a study about metabolite excretion in soldiers deployed to Afghanistan. The vector pressure in this country is so high, that it was concidered an ethical issue to exclude specific soldiers from wearing permethrin impregnated uniforms. Therefore the "controls" were studied before the impregnated BDUs were supplied to the troops in Afghanistan. An exposed group, wearing the impregnated uniform for different periods of time was tested later. Because of its very short half-life and very low exposures expected, permethrin was not determined in plasma or serum: permethrin metabolite levels in urine were measured instead. Spot urine was preferred, so that participation for the soldiers was simple. A specific medical questionnaire was answered; spot urine samples were taken, immediately frozen and transported under stable temperature conditions from Afghanistan to Germany. The permethrin metabolites (3-PBA), cis-DCCA, and trans-DCCA were determined. To exclude confounding exposure to deltamethrin and cyfluthrin (often used for pest control

operations during deployment), their specific metabolites F-PBA and Br_2CA were also determined. Laboratory procedures were conducted according to a procedure modified from Schettgen [27,28]. On the basis of animal studies [17], dermal absorption studies following topical administration [29,46], and the specific procedure of textile treatment by UTEXBel, a very low metabolite concentration had been expected. But the result was different. Whereas metabolite levels of the non-exposed soldiers were comparable to the non-exposed German general population, metabolite levels of the exposed soldiers exceeded these by far, but still well below the ADI (just one soldier with the highest metabolite concentration of all participants was close to 20% of the ADI). Therefore health impairments are rather unlikely.

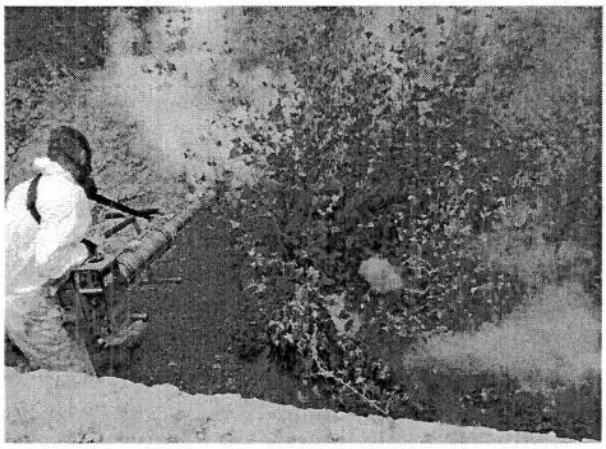

Figure 3: Outdoor permethrin fogging operation during deployment (potential confounding factor)

Investigations showed that during deployment permethrin was used routinely in fogging and spraying operations, which could have confounded the results from the uniform exposure period. Therefore, another study was conducted in Germany under garrison conditions. To exclude confounders like pest control operations, the participating soldiers came from two different garrisons [47]. Exposed soldiers and controls wore BDUs for four consecutive weeks. Spot urine samples were taken before the wearing period, after two and four weeks, and additionally four weeks after the wearing period. Exposed soldiers showed a similar high concentration of permethrin metabolites in urine as those in Afghanistan had shown. These results indicated an exposure to permethrin, when wearing the impregnated uniform. The mean trans-DCCA/cis-DCCA ratio in exposed soldiers was 2.7 [47,48] which could be a sign of a more oral/inhalation exposure [25,29]. Additionally, an elevated metabolite content was found in urine of the exposed soldiers four weeks after exposure. The reason for this is not clear. Compared to the results of other human exposure studies [25,29,46] showing very short metabolite half-life and complete elimination within 150 hours after topical administration, the result of the study needs an explanation. The difference could remain in the exposure times (single/short term exposure vs long time exposure), but further research is needed. Both studies indicate metabolite concentrations far above the reference levels of the general population in Germany, but well below the ADI. Permethrin related health effects were not found [49]. Confounding deltamethrin and cyfluthrin exposures could be excluded in both studies. Both studies have been conducted by the Institute of Occupational, Social and Environmental Medicine of the Johannes-Gutenberg-University Mainz, Germany. Extensive results will be published soon in English. Preanalytics were prepared by Bundeswehr Regional Medical Command II, Occupational and Environmental Medicine, Diez, Germany.

Taking into account the problem of vector-borne diseases during military deployments, the benefit of impregnating BDUs with permethrin is far higher than any potential risk of wearing this BDU.

There are different approaches for estimating pyrethroid exposures during military deployments depending on whether the exposure is more "occupational" or "environmental". The Bundeswehr is studying both types of pyrethroid exposure. Whereas the study described above had an "environmental" background; another study, currently being conducted by the *Bundeswehr Institute of Medical Occupational and Environmental Safety*, is monitoring other "occupational" pyrethroid exposures in addition to those usually expected during deployment.

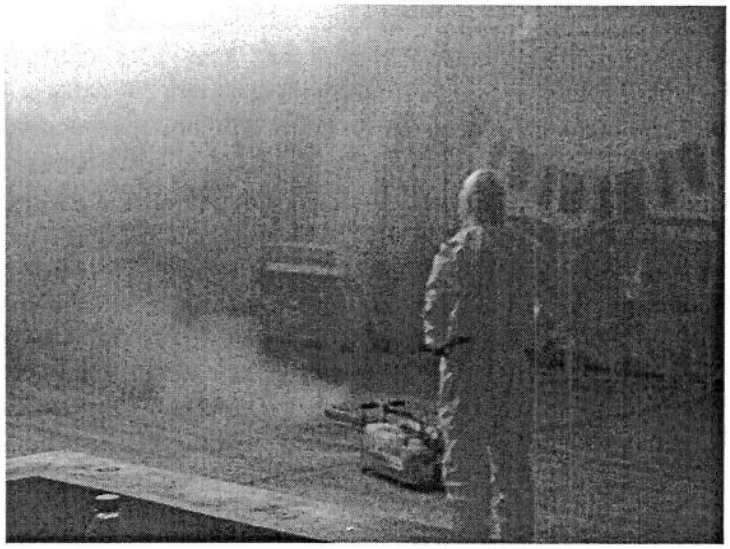

Figure 4: Indoor permethrin fogging operation during deployment (potential confounding factor)

Deployment with a multi-national force brings additional risk of pyrethroid exposure due to several factors. Although the desire to eliminate the vector-borne disease threat in the deployment area is often universal, the pesticides used and application strategies may vary greatly. Additionally, there is the possibility that application of these pesticides may not be coordinated which could result in soldier exposure to a number of chemicals in one treatment area. In those cases, estimating exposures can be misleading if this is not taken into account. Under these conditions, biological monitoring of exposure requires a multilevel approach. Finally, some soldier populations may be further exposed due to their occupational risk, most notably pest control operators, preventive medicine specialists, and those working in NBC defense operations. To observe these soldier populations routinely by biological monitoring of exposure will possibly show additional "occupational" exposures. If elevated metabolite concentrations in urine are found, personal protective equipment must be improved. Therefore, qualified specialists in occupational and environmental health should attend military deployments regularly, to control different paths of exposure and find additional data by conducting questionnaires and taking samples from "occupationally" exposed soldiers. In such cases, it is recommended to collect representative data for long-term assessments, especially when considering the repeated deployments (exposures) of many soldiers.

7.5 CONCLUSION

The healthy and well trained soldier is the most valuable, yet most vulnerable resource an army has. As much as is possible, he must be kept healthy before, during and post deployment. Preventive measures including biomonitoring of exposure are mandatory. Scientific developments in biological monitoring have opened a new world for protection of soldiers. A wide range of chemical substances and their effects on humans is now measurable in the organism. Worldwide military deployments require that this new knowledge base is adopted, to optimize force health protection. To assess the risk of deployed soldiers, monitoring the environment and its influence to soldiers is necessary. Because ambient monitoring and biological monitoring each have their own weaknesses, applying them together in specific areas of exposure provides the best assessment approach of exposure. Strategies of exposure assessment have to be developed and implemented before deployment. Based on actual risk assessments, they should be adjusted before, during, and post deployment, dependent on the specific situation. Furthermore, a rational calculation of the balance between expenditure and benefit should be included in decisions to apply biomonitoring procedures.

Without basic knowledge and voluntary compliance of the soldiers and their leaders, every initiative of a monitoring strategy will be worthless. Although in several armies, under specific exposure conditions, some strategies have been successfully adopted from civilian occupational and environmental medicine, special procedures should be developed which cover the wide range of hazards and the specific living situation of deployed soldiers. Soldiers during deployment are not acting in industrial workplaces with their special standards and techniques. They are moving very rapidly from one adverse environment to another, facing unknown exposures, and are often not aware of imminent dangers. This is of major importance in mixed exposures, especially in situations where chemical, biological, physical, and psychophysical burdens occur in parallel. The scientific development of appropriate technologies and their adoption in military practice is very expensive. It should be managed cooperatively within the scientific military community of NATO to save time, money, and human resources. Civilian research benefiting military applications in the field of biomonitoring should be generously sponsored. For this development to occur, coordinated action and a steady flow of information are essential on national and international levels.

For the military, rapid assessment of exposures will provide soldiers and their military leaders with information critical for preventing or minimizing adverse effects of environmental contaminants, which might possibly degrade the troops readiness significant. Additionally, delayed effects, not readily apparent during a deployment, could cause decreased personnel readiness for future deployments as well as long-term health issues. The cost of these long-term health issues could be substantial. Therefore, the development of methods for the detection of toxic exposures of deployed military personnel is essential. Exposure biomarkers may become a key tool for assessing exposures of military personnel to environmental contaminants during deployments. Knowledge gaps should be defined, research be conducted, and practical field applications be developed, as soon as possible. These field applications should be adjusted continuously to the state of the art of scientific research and the advances in laboratory sciences. Because of costs and benefits, international cooperation is needed. An international standardized toolbox should be introduced in the military area, which makes scientific knowledge easily applicable for use in military operations.

The experiences in biomonitoring procedures concerning permethrin exposures and their sufficient use during military deployments make it a good model for establishing practical tools in the detection of other militarily relevant substances.

At the current state of science, it is unlikely that there will be a single procedure which detects every possible exposure to our military forces. However, individual tests can detect specific exposures and effects. We must consider that unknown factors will continue to pose hazards to our deployed soldiers. Continuing to advance the science of biomonitoring will increase the ability to maintain our soldiers' health and safety in the face of environmental hazards and toxic harmful environments.

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